(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 November 2001 (15.11.2001)

PCT

(10) International Publication Number
WO 01/84961 A2

(51) International Patent Classification[7]: A23L 1/30, 1/302, A23J 7/00

(21) International Application Number: PCT/NL01/00347

(22) International Filing Date: 8 May 2001 (08.05.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/566,386 8 May 2000 (08.05.2000) US
09/703,798 2 November 2000 (02.11.2000) US

(71) Applicant *(for all designated States except US)*: **N.V. NUTRICIA** [NL/NL]; P.O. Box 1, NL-2700 MA Zoetermeer (NL).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **KILIAAN, Amanda, Johanne** [NL/NL]; Harnjesweg 89, NL-6706 AS Wageningen (NL). **HAGEMAN, Robert, Johan, Joseph** [NL/NL]; Hamsterlaan 12, NL-6705 CT Wageningen (NL).

(74) Agent: **JORRITSMA, Ruurd**; Nederlandsch Octrooibureau, Scheveningseweg 82, P.O. Box 29720, NL-2502 LS The Hague (NL).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/84961 A2

(54) Title: PREPARATION FOR THE PREVENTION AND/OR TREATMENT OF VASCULAR DISORDERS

(57) Abstract: The present invention relates to a preparation suitable for the prevention and/or treatment of vascular disorders, comprising the following fractions: fraction a) long chain polyunsaturated fatty acids; fraction b) phospholipids, which fraction contains at least two different phospholipids selected from the group consisting of phosphatidylserine; phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine. fraction c) compounds which are a factor in methionine metabolism, which fraction contains at least one member selected from the group consisting of folic acid, vitamin B12, vitamin B6, magnesium and zinc.

Preparation for the prevention and/or treatment of vascular disorders

Background of the invention

The present invention relates to preparations suitable for the prevention and/or treatment of vascular disorders. The invention also relates to the use of these preparations for the prevention and/or treatment of diseases that find their main cause in vascular problems, in particular dementia syndromes.

The vascular system in the human body is well described in the art. An important part of the system are the blood vessels, that generally are divided in arteries and veins, dependent whether they transport blood to or from the heart. They vary in size from large (e.g. the aorta) to very small (capillaries). From an anatomical point of view larger blood vessels in general comprise as observed from the lumen side:

1. the tunica intima, that consists of a smooth (mono)layer of endothelial cells and a subendothelial layer that consists of a loose layer of connective tissue,
2. the tunica media, which consists of a layer of (innervated) smooth muscle cells and elastic fibers, and
3. the tunica adventitia which is composed of loosely woven collagen fibers, which are infiltrated by tiny lymphatic and blood vessels.

The endothelial cells in the tunica intima are in direct contact with blood and have a barrier function for the underlying tissue. This barrier function includes selective transport of components from blood to the underlying tissue and vice versa, and protection of the underlying tissue. Endothelial cells get easily damaged due to a wide variety of causes like mechanic forces or interaction with stressor components such as classic anaphylatoxins, and components that may occur in the blood, such as homocysteine or components that result from treatment with certain types of drugs (e.g. chemotherapeutics). Vascular permeability can further be increased by a wide variety of humoral- and cell-derived mediators.

Endothelial dysfunction can result in a wide range of disorders. Damage to the endothelial layer can disturb the physiological functions thereof such as transport properties and expose the underlying tissue to stressors. Monocytes may migrate to these damaged spots, get caught by adhesion molecules, differentiate into macrophages, which, when activated, may start up an inflammatory reaction. Due to this reaction cytokines may be released, which may trigger the release of reactive oxygen species, or change coagulation behaviour of blood components. This may result in occurrence of plaques in the arteries, which may ultimately result in hypertension, atherosclerosis and (later) arteriosclerosis.

Atherosclerosis may lead to an impaired blood supply to tissue, which may then become ischaemic. This may lead to damage to cells and even apoptose of the cells that depend on the oxygen and nutrient supply via these blood vessels. Tissue that has become ischaemic may thus lose functional capacity.

Dementia syndromes are characterised by an abnormal high and progressive loss of functional capacity of the brain. This process may start relatively early in life, like the dementias that are associated with some forms of epileptics, encephalitis, Huntington's disease, the dementias that can be observed after intoxication by (chronic) alcohol or drug abuse and the dementias due to cerebrovascular accidents and some genetic forms of dementias (early onset dementias). It may also start relatively late in life such as in Alzheimer's disease (presenile dementia), in senile dementia and in atherosclerotic dementia. Dementias may develop suddenly, e.g. after an apoplectic event, or develop very slowly such as in senile dementia.

Alzheimer's disease is characterised by an early step of excision of the amyloid-beta peptide (AB) from the precursor protein (APP) that is present in the endosomes of neurons or other cells of the central nervous system. The AB peptide produced by beta-secretase (BACE) may diffuse outside the cell and polymerise into amyloid filaments, which in turn may develop into mature amyloid plaques, especially when chaperone molecules like protease inhibitors are present. The inflammatory response to the deposits

of AB polymers and/or amyloid plaque may eventually lead to neuronal cell death and loss of cerebral function.

Dementia syndromes occur relatively frequently; about 10 % of the elderly population in the Netherlands suffer from this disease and still no cure has been found to prevent or treat dementias. Treatment with drugs that increase brain levels of neurotransmitters like acetylcholine, serotonin or (nor)adrenaline are ineffective in the long term and the relatively high doses that often are administered could lead to undesirable side effects.

For the prevention and treatment of vascular disorders no suitable therapy is available either. Vascular disorders and the consequences thereof are a major cause of death in the Western countries. At present vascular disorders are treated by prescribing specific diets that are restricted in cholesterol, saturated fatty acids and in some cases sodium content and by administering drugs that are designed to lower blood pressure (e.g. diuretics), and plasma levels of cholesterol e.g. statins (or other compounds that are able to inhibit the activity of HMG-CoA reductase).

Though some of the treatments are indeed effective in treating part of the phenomena associated with vascular problems, the treatments are not 100% effective in solving the real problem (the cause) and they may demonstrate undesired systemic side effects.

Prior art

Vascular endothelial cells and their function in the blood vessel have been studied for a long time. Many details about biochemical processes that occur in these cells have been published as well.

Recently Chang published in vitro data about the effect of pyridoxal-5-phosphate on human umbilical vein endothelial cells that "suggested that vitamin B6 protects endothelial cells by enhancing the beneficial function and preventing cell injury which are responsible for the initiation and the disease process of atherosclerosis". See Chang S.J.

Nutrition Research, 1999, 19 (11), 1613-1624; "Vitamin B6 protects vascular endothelial injury by activated platelets".

Much literature has appeared about the aetiology of vascular diseases and dementia syndromes. Associations have been made between consumption of fruit on plasma levels of homocysteine or cholesterol and the presence of cardiovascular diseases.

The structure of cell membranes has been studied; many different components appear to be part of the membrane, such as lipids, proteins and cholesterol. Cholesterol appears to be important for the cell membrane. It decreases the fluidity of the outer cell membrane. It also is able to capture some radicals and is claimed to stop self-propagating radical chain reactions in the cell membrane. It is currently thought that vascular disorders should be treated with cholesterol lowering diets. However, it is not recognised that some forms of cholesterol, in particular plasma cholesterol, could be important in the repair mechanisms associated with vascular damage.

WO 99/11625 discloses the use of specific derivatives of huperzine A for inhibiting acetylcholine esterase for example in the treatment of Alzheimer's dementia and myasthemia gravis.

EP 0213724 discloses the use of phosphatidylcholine and phosphatidylethanolamine for membrane fluidisation.

Citric acid and/or citrates are widely used in food manufacture as taste modifier, acidifier and product stabiliser. US 5,234,702 and 5,077,069 disclose the use of citric acid as a synergetic component for the antioxidant action of ascorbyl palmitate, beta-carotene and tocopherol.

WO 99/21565 discloses the use of any Kreb's cycle intermediate selected from citric acid, aconitic acid, isocitric acid, α-ketoglutaric acid, succinic acid, fumaric acid, malic acid

and oxaloacetate or precursors thereof for treating disorders that are characterised by a decreased level of oxidative metabolism (page 5, line 25), in particular disorders of the nervous system but also of cardiovascular diseases. In particular precursors of oxaloacetate are preferred (page 7, line 10). No reference is made to beneficial effects of administration of these components on vasoendothelial cells, nor to additional effects that could be obtained by including phopholipids or LC-PUFA's in the composition.

EP 0 711 559 describes the use of phosphatidylserines for the manufacture of a medicament for improving cerebration, in particular for the treatment of Parkinson' s disease and dementia such as Alzheimer' s disease. The preparation according to this document contains phophatidyl-L-serine which has a structural fatty acid chain derived from at least one raw material lecithin as the effective ingredient. PCT/US88/01693 describes the use of phosphoethanolamine and related compounds for the use in the treatment of Alzheimer's disease.

Summary of the invention

The present inventors have now found a preparation for the treatment of vascular disorders that is effective because it provides activity on the function of the tunica intima and endothelial cells in general, which is important for influencing the aetiology and development of a wide range of vascular disorders and several other secondary disorders, in particular dementia syndromes.

Thus, the present invention provides a preparation suitable for the prevention and/or treatment of vascular disorders, comprising the following fractions:

a) long chain polyunsaturated fatty acids;

b) phospholipids, which fraction contains at least two different phospholipids selected from the group consisting of phosphatidylserine, phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine.

c) compounds which are a factor in methionine metabolism, which fraction contains at least one member selected from the group consisting of folic acid, vitamin B12, vitamin B6, magnesium and zinc or equivalents thereof.

The preparation of the invention can be a pharmaceutical, dietetic as well as a nutritional preparation. The products can have the form of a liquid, powder, bar, cookie, sweetie, concentrate, paste, sauce, gel, emulsion, tablet, capsule, etc. to provide the daily dose of the bioactive components either as a single or in multiple doses. The products can be packaged by applying methods known in the art, to keep the product fresh during shelf life and allow easy use or administration.

Detailed description of the invention

The combined administration of these fractions results in the treatment and prevention of vascular disorders on different levels, in particular on the level of the tunica intima and endothelial cells in general. Fraction a) contains long chain polyunsaturated fatty acids, preferably Ω-3 and/or Ω-6 fatty acids. The fatty acids can be free fatty acids, but are preferably bound to a suitable backbone, for instance a triglyceride. They can also be in the form of phopholipids as will be described later.

The function of fraction a) is to modulate inflammatory processes that may occur in vessel walls and cerebral tissue, to normalise plasma cholesterol levels, especially LDL-cholesterol levels and revert the atherosclerotic process and to increase fluidity of neuronal, erythrocyte and blood vessel membranes. It was found that especially a mixture of Ω-3 and Ω-6 long chain polyunsaturated fatty acids (LCPUFA's) should be included in a ratio of Ω-3 fatty acids to Ω-6 fatty acids of about 2.5 to 5.5 wt/wt.

Preferred Ω-3 LCPUFA's are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Best results are obtained when DHA and EPA are included in about equimolar amounts, for example a ratio of DHA to EPA of 0.5 to 2 wt/wt. Preferred Ω-6 LCPUFA's are dihomogammalinolenic acid (DHGLA) and arachidonic acid (AA). These should be included in an amount of about one fourth of the amount of EPA and DHA, for example a ratio of [DHA + EPA] to [DHGLA + AA] of 2.5 to 5.5, preferably 3.3-4.7 wt/wt. The daily dosage of the total of EPA+DHA+DHGLA+AA is preferably at least 120 mg, more

preferably at least 350 mg. Per daily dose the preparation in particular contains 20 to 2000 mg, preferably 50 to 1000 mg EPA, 50 to 2000 mg, preferably 200 to 1000 mg DHA and 50 to 2000 mg, preferably 100 to 1000 mg DHGLA.

Further LCPUFA's that can be present are linoleic and α-linoleic acid. However, the ratio of the total amount of EPA+DHA+DHGLA+AA to the total amount of linoleic and α-linoleic acid should be larger than 0.1 wt/wt, preferably larger than 0.2, most preferably larger than 0.4.

As described above fraction b) contains at least two different phospholipids selected from the group consisting of phosphatidylserine, phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine. Preferably this fraction contains phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine.

The function of this fraction is to provide a direct source of neuronal and endothelial cell phospholipids. It is highly preferred to include a mixture of phospholipids, especially with regard to the choline/ethanolamine moiety couple on the one hand, and the serine/inositol moiety couple on the other hand. For best results the ratio of (phosphatidylcholine and/or phosphatidylethanolamine) to (phosphatidylserine and/or phosphatidylinositol) is 0.5-20 (wt/wt). Per daily dose at least 0.2 g and preferably more than 1 g phospholipids should be administered, for example 4 g. When the product is meant to be used by patients suffering from dementia syndromes, the amount of phosphatidylserine per daily dose product should be at least 0.1 g for best results and preferably more than 0.5 g.

Another preferred characteristic of the preferred phospholipids is the LCPUFA moiety. It is preferred to use phospholipids which provide the LCPUFA's as described above for fraction a). They can for example be prepared by applying interesterification methods known in the art using for example raw phospholipid mixtures and ingredients that are rich in the particular LCPUFA's. Use of these specific phospholipids ensures a high

activity next to a relatively stable product. In preparations for oral use it is not required to use higher organised lipid fractions such as sphingomyelines due to the high metabolic rates of this type of compound in the gut, gut epithelial cells and liver. Also, other lipids, that are essentially free from DHA, EPA, DHGLA or AA, such as neutral triglycerides are preferably not included in the phospholipid fraction or in relatively low amounts, e.g. less than 40 % and in particular less than 5 % of the lipid fraction. Phospholipids can originate from egg yolk or soy, and can be isolated by applying methods that are known in the art, for example acetone extraction and optionally applying subsequent chromatographic techniques or adsorption methods. The phospholipid fraction can also consist, where required, of mixtures of synthetic phospholipids and (extracts of) phospholipids of natural origin.

Fraction c) contains compounds, which are a factor in methionine metabolism. Total methionine metabolism (TMM) has been described in EP 0 891 719. Though it is known that a proper functioning of TMM is mandatory for the endogenous biosynthesis of many crucial compounds such as S-adenosyl methionine (for creatine, carnitine, etc) and glutathion, and though one has found associations between the occurrence of vascular disorders with hyperhomocysteinaemia, the relevance of a proper functioning of total methionine metabolism for in particular the endothelial cell has not been recognised.

Fraction c consists of compounds which are a factor in methionine metabolism and contains at least one member selected from the group consisting of folic acid, vitamin B12, vitamin B6, magnesium and zinc. Preferably this fraction contains at least folic acid, in particular in an amount of at least 200 μg and most preferably more than 400 μg per daily dose. Folic acid is also meant to include physiological equivalents thereof such as pharmaceutically acceptable salts thereof, 5-methyltetrahydrofolate and polyglutamate forms thereof as occur naturally. It is most preferred that at least folic acid and vitamin B6 are included, while the largest part of the population will benefit if these components are simultaneously included. Vitamin B6 should be included in an amount of more than 2 mg, in particular more than 2.5 mg per daily dose. It is even more advantageous when

this fraction contains all of the members of the above mentioned group. The fraction can further contain SAMe (S-adenosyl methionine), choline, betaine and/or copper. If fraction c) comprises zinc and copper, the weight ratio of zinc to copper is between 5 to 12. Choline and/or betaine can be included.

Besides the fractions a) to c) described above, the preparation according to the invention can contain a further fraction d) that contains citrate. Citrate is also meant to include citric acid. The products according to the invention should have a pH between 3.0 and 7.5 and preferably between 5 and 7. Citrate should be administered in an amount of 0.5 to 30 g, preferably 1.5 to 10 g per daily dose, for example more than 2.4 g.

In the biochemistry literature one can find that citric acid, as well as some other compounds, provides reducing equivalents to the cytosol and participates in the "Krebs cycle", thus yielding NADH and energy in the mitochondriae. It is also known for a long time that citric acid helps regulate glycolyses by feedback inhibition of the phosphofructokinase reaction.

However, it is not recognised that for a proper functioning of vascular endothelial cells it is important to have at the same time sufficient amounts of ATP and reducing equivalents in the form of NADPH available in the cytosol of these cells and that citrate can ensure this to occur, and more effectively than a functional analog like a Krebs cycle intermediate like oxaloacetate, malic acid or fumarate.

It is advantageous to include huperzine A or functional analogues thereof (fraction e), especially in those products that are designed to be used for the prevention and treatment of dementia syndromes, especially in those phases of the disease where acetylcholine metabolism is severely impaired. Huperzine A should be included in amounts of 0.04 to 2, preferably 0.07 to 1, most preferably 0.08 to 0.5 mg per daily dose. As an analogue also an extract of certain herbs such as Huperzia serrata can be used, when standardised on huperzine A content and purity. An amount of 0.04 to 20 mg, preferably 0.07 to 2 mg per daily dose of such an extract can be used. Also lipophilic derivatives of huperzine A

can be used, e.g. those obtained by modification of the primary and/or secondary amino groups.

According to the invention preparations that comprise a fraction a), a fraction b) and a fraction c) as described above together with huperzine and/or citrate are highly preferred since they have in some patients an unexpected higher activity than a product that comprises fraction a, b and c without huperzine and/or citrate.

The preparation preferably further contains a fraction f) one or more of carnitine, vitamin B1, vitamin B5 and coenzyme Q10 or functional analogues thereof. As functional equivalents of carnitine can be mentioned pharmaceutically acceptable salts thereof or alkanoyl and acyl carnitines [acetyl-L-carnitine], which are particularly useful, or mixtures thereof. Carnitine is advantageously included in products that are meant to be used for patients suffering from dementia syndromes. In these products preferably a lipophilic derivative is used as carnitine source. It is most preferred to use acetyl-L-carnitine. This component provides acetyl groups in the brain for biosynthetic purposes. Carnitine should be included in an amount of 0.1 to 3 g, preferably 0.2 to 1 g per daily dose. Vitamin B5 can be included for instance as calcium pantothenate or other stable form. Preferred dosages are 8 to 80 mg, preferably 12 to 40 mg per daily dose product.

For products that are meant to be used for treatment or prevention of further progression of dementia syndromes it is preferred to use a lipophylic thiamine source such as benfothiamine, allithiamine, fursulthiamine or octothiamine. A degeneration of cerebral function as is observed during Parkinson's and Huntington's disease can be retarded by the product according to the invention. In products for these types of patients it is advantageous to include also respectively taurine and gamma-amino butyric acid or derivatives thereof such as piracetam. If coenzym Q10 is included the amount can be 0.8 to 200 mg and preferably 5 to 70 mg. The amounts can be that low because of the beneficial effect of the phospholipids on the membrane function.

Also a fraction g) can be present that provides anti-oxidant properties. Fraction g) contains antioxidants selected from vitamin C, vitamin E, lipoic acid, selenium salts and carotenoids. A fraction h) contains an extract of gingko biloba. This extract is obtained from the leaves and is enriched in flavonoids and especially terpenoids, in particular ginkgolides. It appears for example that an extract that comprises at least 4 % ginkgolides is effective.

The preparation preferably contains the above components of the different fractions in an amount above the recommended daily intake. Per daily dose the preparation of the invention preferably comprises:

at least 120 mg long chain polyunsaturated fatty acids;

at least 200 mg phospholipids;

at least 200 µg folic acid; and

at least 0.5 g citrate.

More preferably, the preparation comprises per daily dose:

at least 20 mg, preferably at least 50 mg eicosapentaenoic acid

at least 50 mg, preferably at least 200 mg docosahexaenoic acid

at least 50, mg preferably at least 100 mg arachidonic acid

at least 200 mg, preferably at least 1000 mg phosphatidylserine,

at least 200 µg, preferably at least 400 µg folic acid

at least 100 mg, preferably at least 200 mg magnesium

at least 5 mg, preferably at least 10 mg zinc

at least 2 mg, preferably at least 2.5 mg vitamin B6

at least 2 µg, preferably at least 4 µg vitamin B12

at least 1.0 g, preferably at least 1.5 g citrate.

The preparations according to the invention can be used in the treatment and/or prevention of vascular, cardio- and cerebrovascular disorders and a selected range of secondary problems. The nature and impact of the latter depends on the time pattern and degree of

decrease of the blood flow and the function of the organ/ tissue that is involved. Damage to the endothelial cells may also lead to loss of elasticity and even a local lesion of the blood vessel.

Examples of problems of the cardiovascular system are atherosclerosis obliterans, angina pectoris, myocard infarct, cerebral vascular accidents, thrombosis, M. Bürger, varices, thrombo-phlebitis and the syndrome of Raynaud. Damage to endothelial cells may also lead to a localized vasoconstriction. When damage to the endothelial cells has a more systemic nature this may lead to increased blood pressure. Other vascular disorders that could be treated with the product are atherosclerosis, arteriosclerosis, hypercholesterolaemia, hyperlipidaemia, elevated blood pressure, angina pectoris, cerebrovasular accidents, temporary disorders associated with ischaemia, vene thrombose, postpartum thrombose, myocard infarct, varicose veins (varices), thrombo angiitis obliterans and atheroslecrosis obliterans.

Secondary problems that are treatable with the product are some problems that occur after mechanic traumata, such as those that occur in accidents, during or after labor and during surgery. Other examples of treatable secondary problems are problems with hearing loss (especially that associated with chemotherapy or ageing), with improper functioning of liver, stomach, kidneys, legs, lung, or prostate and sudden (cerebrovascular incident) or chronic decrease of cerebral functions. An especially preferred use is in the prevention of cognitive degeneration in persons at risk for Alzheimer's disease or vascular dementia.

We believe that many of the dementia syndromes find their basic cause in cardiovascular disorders. An effective blood supply would provide sufficient amounts of nutrients to the brain, which supports anti-inflammatory response to deposits of AB peptide polymer and helps degrade plaques. An adequate blood supply further can help prevent the degenerative oxidative processes that are observed in many neurological disorders such as in Parkinson's disease (in the substantia nigra) and supports biosynthesis and metabolism of neurotransmitters such as gamma-amino butryric acid and dopamine.

Example 1

Capsule for use by demented persons three times a day.

The capsule is prepared using methods known in the art and comprises as active components:

DHA	50 mg
EPA	75 mg
phospholipids*	250 mg
folic acid	200 µg
vitamin B12	25 mg
Huperzia serrata	100 ug
vitamin B1	100 mg
coenzym Q10	10 mg
vitamin E	200 mg
Gingko biloba	120 mg

* phosphatidylcholine 130 mg, phosphatidylserine 120 mg (synthetic)

Example 2

Pudding for improvement of vascular conditions and secondary disorders such as dementia syndromes. The pudding is based on cow's milk, starch, sugar and flavourings prepared by methods known in the art and comprises as active ingredients per serving of 200 ml:

phospholipids from egg	4 g
(provides about 20 mg DHA and 20 mg AA and phospholipids comprise about 77 % phosphatidylcholine and 16 % phosphatidylethanolamine)	
phosphatidylserine	100 mg
encapsulated fish oil	0.3 g
(provides about 30 mg DHA and 30 mg EPA)	
Single cell oil (25 mg AA)	0.06 g
folic acid (synthetic monoglutamate)	0.3 mg
vitamin B6 (pyridoxine)	3 mg

zinc (ZnSO4)	10 mg
magnesium (MgO)	100 mg
vitamin B12 (cyanocobalamine)	3 µg
citrate/ citric acid	1.2 g
thiamine HCl	2 mg
vitamin E	20 mg
manganese (MnO)	10 mg

Example 3

Powdered concentrate that consists of the active components as given in example 2 and further 0.1 g orange flavouring and maltodextrine to make up a total mass of 10 g. This amount is packed in a sachet and can be used to be reconstituted in any drink (milk, fruit juice, etc.).

Example 4

Powder for improvement of vasoendothelial function consisting of

soylecithin*	3 g
folic acid	400 µg
vitamin B6	3 mg
vitamin B12	4 µg
zinc	15 mg
magnesium	150 mg
citric acid/ citrate (pH of product 7.0)	2.2 g
maltodextrines	to make up a total weight of 10 g

* phosphatidylcholine:phosphatidylethanolamine:phosphatidylinositol = 24:22:15)

Example 5

Muesli-bar of about 25 g based on sugar, cereals and pieces of dried fruit that comprises as active components:

soylecithin*	2 g

encapsulated fish oil	0.6 g
SCO (AA)	0.3 g
Folic acid	400 µg
pyridoxamine	3 mg
cyanocobalamine	5 µg
zinc oxide	30 mg
magnesium oxide	200 mg
citric acid/citrate pH 6.5 mixture	2 g
Huperzine serrata extract	150 µg
Gingko biloba extract	200 mg
calcium sulphate	300 mg
vitamin D	10 µg

* phosphatidylcholine:phosphatidylethanolamine:phosphatidylinositol = 45:26:14)

The bar is coated with a layer of chocolate.

Claims

1. Preparation suitable for the prevention and/or treatment of vascular disorders, comprising the following fractions:

a) long chain polyunsaturated fatty acids;

b) phospholipids, which fraction contains at least two different phospholipids selected from the group consisting of phosphatidylserine, phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine.

c) compounds which are a factor in methionine metabolism, which fraction contains at least one member selected from the group consisting of folic acid, vitamin B12, vitamin B6, magnesium and zinc or equivalents thereof.

2. Preparation according to claim 1, further comprising a fraction d) citrate or citric acid.

3. Preparation according to claim 1 or 2, further comprising a fraction e) huperzine A or functional analogs thereof.

4. Preparation according to any of claims 1 to 3, wherein fraction a) comprises Ω-3 and Ω-6 fatty acids.

5 Preparation according to claim 4, wherein the Ω-3 fatty acids are selected from the group consisting of eicosapentaenoic acid and docosahexaenoic acid and the Ω-6 fatty acids are selected from the group consisting of arachidonic acid and dihomogammalinolenic acid.

6. Preparation according to any of claims 1 to 5, wherein fraction b) comprises phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine.

7. Preparation according to any of claims 1 to 6, wherein fraction c) contains at least folic acid and vitamin B6.

8. Preparation according to any of claims 1 to 7, wherein fraction c) further contains SAMe, choline, betaine and/or copper.

9. Preparation according to claim 8, wherein fraction c) comprises zinc and copper, wherein the weight ratio of zinc to copper is between 5 to 12.

10. Preparation according to any of claims 1 to 9, which further contains a fraction f) one or more members selected from the group consisting of carnitine, vitamin B1, vitamin B5 and coenzyme Q10 or functional analogues thereof.

11. Preparation according to any of claims 1 to 10, which further contains a fraction g) one or more antioxidants selected from vitamin C, vitamin E, lipoic acid, selenium salt and carotenoids.

12. Preparation according to any of claims 1 to 11, which further contains a fraction h) an extract of gingko biloba.

13. Preparation according to any of claims 1 to 12, which comprises per daily dose:
at least 120 mg of long chain polyunsaturated fatty acids;
at least 200 mg phospholipids;
at least 200 µg folic acid; and
at least 500 mg citrate.

14. Preparation according to claim 13, which comprises per daily dose:
at least 20 mg, preferably at least 50 mg eicosapentaenoic acid
at least 50 mg, preferably at least 200 mg docosahexaenoic acid
at least 50 mg, preferably at least 100 mg arachidonic acid
at least 200 mg, preferably at least 1000 mg phospholipids,

at least 200 µg, preferably at least 400 µg folic acid
at least 100 mg, preferably at least 200 mg magnesium
at least 5 mg, preferably at least 10 mg zinc
at least 2 mg, preferably at least 2.5 mg vitamin B6
at least 2 µg, preferably at least 4 µg vitamin B12
at least 1.0 g, preferably at least 1.5 g citrate.

15. Use of a preparation according to any of claims 1 to 14 for the treatment and/or prevention of vascular disorders or secondary disorders associated therewith.

16. Use according to claim 15, wherein the vascular disorder is atherosclerosis, arteriosclerosis, hypercholesterolaemia, hyperlipidaemia, elevated blood pressure, angina pectoris, dementia syndromes, cerebrovasular accidents, temporary disorders associated with ischaemia, M. Raynaud, vene thrombose, postpartum thrombose, myocard infarct, varicose veins, thrombo angiitis obliterans and atheroslecrosis obliterans.

17. Use according to claim 15, wherein in the secondary vascular disorder is dementia syndromes, cognitive degeneration or hearing loss.

18. Preparation according to claim 1, which is a nutritional supplement.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 November 2001 (15.11.2001)

PCT

(10) International Publication Number
WO 01/084961 A3

(51) International Patent Classification[7]: A23L 1/30, 1/302, A23J 7/00, A23L 1/304

(21) International Application Number: PCT/NL01/00347

(22) International Filing Date: 8 May 2001 (08.05.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/566,386 8 May 2000 (08.05.2000) US
09/703,798 2 November 2000 (02.11.2000) US

(71) Applicant *(for all designated States except US)*: **N.V. NUTRICIA** [NL/NL]; P.O. Box 1, NL-2700 MA Zoetermeer (NL).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **KILIAAN, Amanda, Johanne** [NL/NL]; Harnjesweg 89, NL-6706 AS Wageningen (NL). **HAGEMAN, Robert, Johan, Joseph** [NL/NL]; Hamsterlaan 12, NL-6705 CT Wageningen (NL).

(74) Agent: **JORRITSMA, Ruurd**; Nederlandsch Octrooibureau, Scheveningseweg 82, P.O. Box 29720, NL-2502 LS The Hague (NL).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
15 August 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/084961 A3

(54) Title: PREPARATION FOR THE PREVENTION AND/OR TREATMENT OF VASCULAR DISORDERS

(57) Abstract: The present invention relates to a preparation suitable for the prevention and/or treatment of vascular disorders, comprising the following fractions: fraction a) long chain polyunsaturated fatty acids; fraction b) phospholipids, which fraction contains at least two different phospholipids selected from the group consisting of phosphatidylserine; phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine. fraction c) compounds which are a factor in methionine metabolism, which fraction contains at least one member selected from the group consisting of folic acid, vitamin B12, vitamin B6, magnesium and zinc.

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23L1/30 A23L1/302 A23J7/00 A23L1/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23L A61K A23J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, FSTA, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	EP 0 721 742 A (CLINTEC NUTRITION CO) 17 July 1996 (1996-07-17) claims 1,3 page 2, line 36-43 page 3, line 1-3 page 4, line 4-9,15-55 page 5, line 14-18 ---	1,4,7,8, 10,11,18 2,3,5,6, 9,12-17
X A	WO 94 27628 A (ABBOTT LAB) 8 December 1994 (1994-12-08) claims 1,2,5; tables 3,4,6 page 7, paragraph 1 ---	1,2,4, 7-11,18 3,5,6, 12-17
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 December 2001	10/12/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Tallgren, A

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, [redacted]cation,where appropriate, of the relevant passages [redacted]	Relevant to claim No.
X A	WO 97 39749 A (ABBOTT LAB) 30 October 1997 (1997-10-30) claims 1,4,6; examples 1,3,4; tables 2,3,6,7 page 10, line 4-19	1,2,4,5, 7-11,18 3,6, 12-17
X A	WO 93 19624 A (ABBOTT LAB) 14 October 1993 (1993-10-14) claims 1-10; tables 2-8 page 8, paragraphs 3,4	1,2,4,5, 7-11,15, 16,18 3,6, 12-14,17
X	FR 2 773 484 A (MOREAU PIERRE) 16 July 1999 (1999-07-16) claims 1,6-8,10,12,13,18; example 4 page 4, line 37-39 page 5, line 26-37	1,2,4,5, 10-12, 15,16,18
X	EP 0 652 012 A (NAITO ALBERT) 10 May 1995 (1995-05-10) claim 3; example 16	1,4,7,8, 10,11, 15,17,18

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
EP 0721742	A	17-07-1996	US	5589468 A	31-12-1996
			AU	4076595 A	25-07-1996
			CA	2166003 A1	14-07-1996
			EP	0721742 A1	17-07-1996
			JP	8231411 A	10-09-1996
			US	RE37020 E1	16-01-2001
			US	RE36288 E	31-08-1999
			US	5686429 A	11-11-1997
WO 9427628	A	08-12-1994	CA	2162042 A1	08-12-1994
			EP	0700303 A1	13-03-1996
			WO	9427628 A1	08-12-1994
			US	5700782 A	23-12-1997
WO 9739749	A	30-10-1997	US	6077828 A	20-06-2000
			AU	716532 B2	24-02-2000
			AU	2742497 A	12-11-1997
			CA	2252513 A1	30-10-1997
			EP	0914111 A2	12-05-1999
			JP	11508282 T	21-07-1999
			WO	9739749 A2	30-10-1997
WO 9319624	A	14-10-1993	US	5223285 A	29-06-1993
			AU	3614693 A	08-11-1993
			DE	69329201 D1	14-09-2000
			DE	69329201 T2	12-04-2001
			EP	0656754 A1	14-06-1995
			ES	2150440 T3	01-12-2000
			JP	8011048 B	07-02-1996
			JP	7501457 T	16-02-1995
			MX	9301787 A1	28-02-1994
			NZ	249397 A	26-01-1996
			WO	9319624 A1	14-10-1993
FR 2773484	A	16-07-1999	FR	2773484 A1	16-07-1999
EP 0652012	A	10-05-1995	CA	2103399 A1	19-05-1995
			EP	0652012 A1	10-05-1995